ORIGINAL ARTICLE INFECTIOUS DISEASE

Association between serological evidence of past *Coxiella burnetii* infection and atherosclerotic cardiovascular disease in elderly patients

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Abstract

Q fever, caused by *Coxiella burnetii*, may cause vascular complications, but the role that this infection may play in the development of atherosclerotic cardiovascular disease remains unknown. This study examined the association between Q fever serology and cardiovascular disease in a region where Q fever is endemic. A case-control study was conducted in the Hospital Universitario de Burgos (Spain) between February 2011 and June 2012. A total of 513 samples were tested, from 454 hospitalized patients ≥65 years old, of whom 164 were cases (patients with prevalent or incident coronary heart, cerebrovascular or peripheral artery, disease) and 290 controls (patients without cardiovascular disease). Serum IgG antibody phase II titres against Q fever were determined by immunofluorescence assay. Seropositivity (titres ≥1:256) was detected in 84/164 (51.2%) cases and in 109/290 (37.6%) controls ($p = 0.005$; OR, 1.7; 95% CI, 1.1–2.5). This ratio increases when adjusted for sex, hypertension, dyslipidaemia, smoking, diabetes and atrial fibrillation (OR, 2.6; 95% CI, 1.5–4.7). The geometric mean titre (GMT) for *C. burnetii* phase II assay was higher in cases than in controls ($p = 0.004$). We found no significant relationship between cardiovascular disease and *C. pneumoniae*, and Cytomegalovirus seropositivity (both determined by the IgG ELISA method). In conclusion, serological evidence of past Q fever is associated with atherosclerotic cardiovascular disease in elderly patients in an endemic region.

Keywords: Atherosclerotic disease, cardiovascular disease, *Coxiella burnetii*, Q fever, Q fever serology
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Introduction

Coxiella burnetii is a strict intracellular pathogen causing Q fever, a worldwide zoonosis with an extensive animal reservoir [1–3]. Infected animals suffer from spontaneous abortions and other obstetric complications. In humans, it can cause a self-limited flu-like illness, pneumonia and hepatitis, but a large proportion of cases are asymptomatic [1–3]. Chronic Q fever infections are frequently associated with cardiovascular complications [4], mainly endocarditis [3], and also aortic aneurysms and vascular-graft infection [4,5]. Serology is the cornerstone of diagnosis, and immunofluorescence assay is the current reference method for the serodiagnosis of Q fever [1].

Persistent infection may contribute to the pathogenesis of atherosclerosis. *Chlamydia pneumonia* and Cytomegalovirus (CMV) are possibly the microorganisms most studied, but many others may be involved [6]. *Coxiella burnetii* is a plausible candidate, because in addition to the above, it can cause myocardial infarction [7], myocarditis [1], stroke [4], and thrombus and arterial embolism [8]. As regards these data, some authors have investigated whether there is an association between past infection due to *C. burnetii* and cardiovascular disease in middle-aged subjects. However, the results so far have been controversial [9–13]. This relationship has never been studied in the elderly population, in whom the prevalence of both diseases (cardiovascular disease and Q fever) is higher than in middle-aged subjects. The latter is especially evident in

endemic areas of Europe, where seroprevalence in the elderly can reach 70% [14].

To clarify this issue, the association between Q fever serology and cardiovascular disease was analysed in elderly patients in a highly endemic region.

Methods

Patients and sample selection criteria

A case-control study was conducted in the Hospital Universitario de Burgos (Burgos area, Spain) between February 2011 and June 2012. This centre is the only public hospital in its area, which covers around 200 000 inhabitants. A total of 513 samples were tested, from 454 hospitalized patients ≥65 years old, of whom 164 were cases and 290 controls. The study protocol was reviewed and approved by the Clinical Research Ethics Committee of this hospital, and the patients signed an Informed Consent Form (adapted to the Declaration of Helsinki principles).

The cases were those who had prevalent or incident atherosclerosis cardiovascular disease, defined as coronary heart disease, manifested by myocardial infarction or angina pectoris, and/or cerebrovascular disease, manifested by stroke and transient ischaemic attack, and/or peripheral artery disease, manifested by intermittent claudication to limb-threatening ischaemia [15]. Heart failure and brain haemorrhage were not included in the definition of the case group. The presence of atrial fibrillation, or other known source of cardiac emboli (valvular prosthesis, valvulopaties), was not cause of exclusion from the case group. This is because the presence of a known source of cardiac emboli does not always mean that there is a causal relationship [16]. Indeed, thromboembolism of aortic or large vessels atheroma should be considered in these patients [16]. Cardiovascular disease caused by endocarditis, intracardiac tumors, hypercoagulable state, vasculitides and other non-atherosclerotic diseases, was included in the control group. The controls showed no history or clinical evidence of atherosclerotic cardiovascular disease. The sample was recruited at a rate of approximately two patients per day, randomly selected using a computer program.

Data collection and cardiovascular risk factors

Only the histories that could be verified from a medical report or during hospitalization were taken into account. Atherosclerotic cardiovascular disease was only considered valid if it was supported by imaging techniques or, if these were not available, was verified by a cardiovascular specialist. None of the physicians who classified the patients knew the serological results beforehand. Furthermore, our laboratory was not aware of the source of the samples, nor did it have any clinical information about them.

The control variables used were age, sex, hypertension, diabetes, dyslipidaemia, atrial fibrillation/atrial flutter (sustained or paroxysmal) and smoking. It is unknown whether the association at issue may be influenced by any of these variables, so we decided not to match cases and controls for any of them. A smoker was someone who was known to have smoked continuously (several years) at any time in their life. We did not formulate any other definition due to the difficulty of obtaining more precise information. Serum C- reactive protein was determined, as an acute or chronic inflammation may result in endothelial dysfunction, and may be responsible for a cardiac event [17]. The co-morbidities were standardized according to the Charlson criteria [18].

Serological determinations

Q fever serology was determined by immunofluorescence assay using reagents and test protocols from Focus Technologies (formerly MRL, Cypress, CA, USA). Blood samples were collected by venepuncture, and serum samples were stored at −20°C until analysis. IgG phase II titres equal to or >1:256, were considered positive. Low titres of IgG phase II usually indicate a past infection, but with high titres, an acute infection cannot be ruled out [1–3]. The decision to extend the serology study (IgM antibodies), or to rule out an acute or chronic infection, was based on clinical criteria, and was the responsibility of an experienced internal medicine physician. The geometric mean titre (GMT) was also calculated in order to evaluate the seropositivity rate. An IgG phase II titre <1:256 was assigned a value of 1:64. To check the serological test precision, a second serological determination was performed 2–3 days after the first one in 41 patients. The agreement of the results was 100% as regards seropositivity, and 97.5% agreement for titres.

The same blood sample was used to determine the presence of IgG antibody to *Chlamydia pneumoniae* and CMV (using commercial enzyme-linked immunosorbent assay (ELISA) kits), C-reactive protein (CRP), albumin and lipid profile (total cholesterol, LDL and HDL cholesterol, and triglycerides), as well as other biochemistry and haematology parameters. An index >0.9 for *C. pneumoniae* and >70 for CMV was considered positive. The comparison of indices (Mann–Whitney *U*-test) was performed, assigning a value of 0.5 for *C. pneumoniae* negative values, and a value of '0' for CMV negative values.

Determination of sample size and statistical analysis

Given the prevalence of Q fever in regions adjacent to ours [14], and expecting to see an odds ratio (OR) of 2.5 or more, with an α risk of 5% and β of 10% (90% power of the study), and a two-tailed hypothesis, the minimum number of cases to

study should be 112. Logistic regression analysis was used to test for a possible relationship between seropositivity to *Coxiella burnetti* and cardiovascular disease. The data were analysed using the statistics program, SPSS 15.0 (SPSS, Chicago, Illinois, USA). The OR was calculated with a confidence interval of 95%. The statistical tests were considered significant for values of $p < 0.05$.

Results

Serum was available for serology in all patients. Two patients with a history of transient ischaemic attack were included in the control group after being unable to verify with certainty these diagnoses. We have not found cardiovascular disease caused by endocarditis, hypercoagulable state, vasculitides or other non-atherosclerotic diseases. The epidemiological and clinical characteristics of the cases and the controls are summarized in Table 1. Except for diabetes, the rest of the cardiovascular risk factors behaved as expected. Both groups were similar in terms of major causes of admission and co-morbidities. There were only 15 (9.1%) incident cases of cardiovascular disease, of which 52.2% were seropositive. Seropositive results were found in 53.8% of the 78 patients who suffered from cerebrovascular disease, 50.0% of the 83 with ischaemic heart disease, 52.3% of the 44 with peripheral arterial disease, and in 51.0% of the 41 with mixed cardiovascular disease.

Unlike what happens for *Coxiella* (Table 2), no significant differences were found in the geometric mean values obtained for *Chlamydia* (1.20 (range, 0.5 to 3.3), and 1.20 (range, 0.5 to 4.7); p 0.13) and CMV (53.0 (range, 0 to 400), and 56.0 (range, 0 to 270); p 0.13) in cases and controls. A study was conducted to rule out Q fever endocarditis in two patients with compatible serology and refractory heart failure. Trans-thoracic and trans-oesophageal ultrasound, as well as the PCR assays in blood, were negative in both cases. Twelve patients had acute respiratory infection of atypical characteristics, but none of them had positive IgM antibodies, and all had satisfactory outcomes with conventional therapy.

The distribution of cardiovascular risk factors, co-morbidity and cardiovascular diseases according to the results of serology is shown in Table 3. Only cerebrovascular disease is associated with seropositivity. The unadjusted and adjusted relationships between Q fever seropositivity, *Chlamydia* and CMV seropositivity, and case-control status, are shown in Table 4. Neither the CRP nor age was included in the regression analysis. The first due to being similar in both groups and second because it is a variable subject to control.

TABLE 1. Characteristics of cases and controls

	Cases (n = 164)	Controls (n = 290)	p value
Age	81.3 (7.6)	80.7 (7.7)	0.45
Men	114 (69.5)	129 (44.5)	<0.001
Caucasian ethnicity	163 (99.3)	288 (99.3)	1.00
Diabetes	55 (33.5)	83 (28.6)	0.55
Hypertension	131 (79.9)	189 (65.2)	0.001
Dyslipidaemia	87 (53.0)	106 (36.6)	0.001
Atrial fibrillation	85 (51.8)	103 (35.5)	0.001
Smoking	84 (51.2)	94 (32.4)	<0.001
Ex-smoker	78 (47.6)	84 (29.0)	<0.001
Current smoker	4 (2.4)	9 (3.1)	0.76
C-reactive protein (mg/L)	53.7 (72.3)	57.2 (71.2)	0.66
Albumin (mg/dL)	2871.4 (612.1)	2839.1 (632.6)	0.54
Other co-morbidities[a]			
Congestive heart failure	57 (34.8)	80 (27.6)	0.11
Neoplasm (any) [b]	38 (32.2)	71 (24.5)	0.81
Chronic pulmonary disease	52 (31.7)	70 (24.1)	0.09
Dementia	23 (14.0)	45 (15.5)	0.78
Moderate or severe renal disease	7 (4.3)	7 (2.4)	0.27
Liver disease	4 (2.4)	12 (4.1)	0.32
Connective tissue disease	3 (1.8)	8 (2.8)	0.75
Causes of hospitalization			
Respiratory diseases	46 (28.0)	79 (27.2)	0.95
Gastrointestinal diseases	28 (17.1)	67 (23.1)	0.15
Other cardiovascular diseases	23 (14.0)	44 (15.1)	0.87
Urological diseases	14 (8.5)	28 (29.7)	0.73
Locomotor diseases	12 (7.3)	34 (11.7)	0.14
Haematological disease	4 (2.4)	8 (2.8)	0.55
Skin or soft tissue diseases	3 (1.8)	9 (3.1)	0.55
Other diseases	12 (7.3)	21 (7.2)	1.00

Quantitative variables expressed as median and standard deviation. Dichotomous variables expressed in number and (%); Mann–Whitney *U* or Fisher's exact chi-squared test (two-sided), according to the type of variable; Cases, patients with prevalent or incident coronary heart, cerebrovascular or peripheral artery disease; Controls, patients without cardiovascular disease.
[a]The co-morbidities were standardized according to the Charlson criteria [18].
[b]Haematological tumors included.

TABLE 2. Distribution of serological IgG phase II titres against *Coxiella burnetii* in study patients

	Cases (n = 164)	Controls (n = 290)	p value
<1:256	80 (48.8)	181 (62.4)	–
≥1:256	84 (51.2)	109 (37.6)	0.006
≥1:512	22 (13.4)	24 (8.3)	0.10
≥1:1024	9 (5.5)	8 (2.8)	0.10
≥1:2048	6 (3.7)	3 (1.0)	0.07
			0.004[a]

Expressed as (%), and p value obtained by the Fisher's exact chi-squared test (two-sided); Cases, patients with prevalent or incident coronary heart, cerebrovascular or peripheral artery disease; Controls, patients without cardiovascular disease.
[a]p value obtained by Mann–Whitney *U* for *C. burnetii* phase II (geometric mean titre 248.6 (range, 64 to 2048) and 215.5 (range 64 to 2048), respectively).

The odds of Q fever seropositivity were elevated, and increased after adjusting for covariates and significant interactions between covariates and the main variable being studied (Table 4). Except for hypertension, which produced a negative interaction, the rest of the control variables were involved in positive interactions. However, only the significant interactions were included in the regression model ($p < 0.05$). Also, none of the cardiovascular risk factors acted as a confounding factor when considering them individually. No interactions were found for *Chlamydia* and CMV seropositivity (Table 4),

TABLE 3. Characteristics of patients according to their *Coxiella burnetii* serostatus

	Seropositives (*n* = 193)	Seronegatives (*n* = 261)	p value
Cardiovascular risk factors			
Age	81.2 (7.7)	80.7 (7.6)	0.46
Men	111 (57.5)	132 (45.6)	0.15
Diabetes	62 (32.1)	76 (29.1)	0.53
Hypertension	139 (72.0)	181 (69.3)	0.58
Dyslipidaemia	87 (45.1)	106 (40.6)	0.38
Atrial fibrillation[a]	89 (46.1)	99 (37.9)	0.08
Smoking	84 (43.5)	94 (36.0)	0.14
Atherosclerotic cardiovascular disease			
Coronary heart disease	42 (21.8)	42 (16.1)	0.14
Cerebrovascular disease	42 (21.8)	36 (13.8)	0.03
Peripheral artery disease	23 (11.9)	21 (8.0)	0.20
Other cardiovascular diseases			
Cardiac valvulopathies	33 (17.1)	36 (13.8)	0.35
Valvular prosthesis	8 (4.1)	11 (4.2)	1.00
Vascular grafts	7 (3.6)	5 (1.9)	0.40
Other co-morbidities[b]			
Congestive heart failure	68 (35.2)	69 (26.4)	0.06
Any tumour[c]	46 (23.8)	63 (24.1)	1.00
Chronic pulmonary disease	57 (29.5)	65 (24.9)	0.28
Dementia	32 (16.6)	36 (13.8)	0.42
Moderate or severe renal disease	5 (2.6)	9 (3.4)	0.78
Liver disease	9 (4.6)	7 (2.5)	0.47
Connective tissue disease	3 (1.6)	8 (3.1)	0.38

Quantitative variables expressed as median and standard deviation. Dichotomous variables expressed in number and (%); Mann–Whitney *U* or Fisher's exact chi-squared test (two-sided), according to the type of variable; Cases, according to the type of variable.
IgG phase II titres ≥1:256, determined by immunofluorescence assay, were considered seropositive.
[a]Atrial fibrillation/atrial flutter (sustained or paroxysmal).
[b]The co-morbidities were standardized according to the Charlson criteria [18].
[c]Haematological tumors included.

TABLE 4. Unadjusted and adjusted relationships between seropositivity and cardiovascular disease in study patients

	Cases (*n* = 164) %	Controls (*n* = 290)	Crude OR	Adjusted OR[a]
Coxiella burnetti	51.2	37.6	1.7 (1.1–2.5)	2.6 (1.5–4.7)
Chlamydia pneumoniae	83.5	75.4	1.7 (1.0–2.9)	1.5 (0.9–2.6)
CMV	93.3	96.2	0.5 (0.2–1.2)	0.7 (0.3–2.0)
Coxiella burnetti + *Chlamydia*	43.3	28.6	1.9 (1.2–2.8)	2.8 (1.5–5.0)
Coxiella burnetti + *Chlamydia* + CMV	41.5	26.9	1.9 (2.2–2.8)	2.9 (1.6–5.2)

OR, odds ratio (95% confidence interval); Cases, patients with prevalent or incident coronary heart, cerebrovascular or peripheral artery disease; Controls, patients without cardiovascular disease; CMV, Cytomegalovirus.
[a]Adjusted for sex, hypertension (yes or no), dyslipidaemia (yes or no), smoking habits (yes or no), diabetes (yes or no), atrial fibrillation (yes or no), and significant interactions between the covariates and the primary endpoint: *Coxiella* seropositivity with smoking (p = 0.04). *Coxiella* + *Chlamydia* seropositivity with smoking (p = 0.01). *Coxiella* + *Chlamydia* + CMV seropositivity with smoking (p = 0.01).

although the seroprevalence of the latter was so high that it invalidated any possible analysis. Although it was not our intention to build a predictive model of cardiovascular disease, we observed that the best model achieved for this purpose only correctly classified 70% of the patients. This means that other covariates (not included in the study) could affect the results through new interactions or by acting as confounding variables.

Discussion

Original tentative suggestions of a link between Q fever and cardiovascular disease were in the form of a case report of acute myocardial infarction in a patient with Q fever infection [6]. More recently, Ender *et al.* performed serology on 55 patients subjected to coronary angiography. The geometric mean titre for *C. burnetti* phase I was slightly higher in persons with coronary artery disease than in those without this disease ($p < 0.02$) [9]. Further positive relationships were reported in a follow-up study of people infected during a large outbreak of Q fever in Switzerland in 1983 [10]. In this study, the patients who had acute Q fever not only had a higher risk of suffering from a cerebrovascular accident and ischaemic heart disease after 12 years, but their mortality was also higher at the end of this time. However, this study was criticized for not controlling for current and past cigarette smoking as a potential explanation for the excess cardiovascular morbidity and mortality observed [11]. Later, a nested case-control study conducted on 335 patients was unable to find any relationship between evidence of past *C. burnetti* infection and incident ischaemic disease, despite taking smoking and other potential confounding factors into account [12]. The inability to find a clear link between these two variables could have been due to the low seroprevalence of the infection in the populations studied. As we have shown, the application of specific serological markers in populations at high risk of Q fever, improves the results obtained.

Although previous studies have suggested an association between smoking status and Q fever, we have observed that the data existing up to now are contradictory and are unable to support this hypothesis. In a large outbreak of Q fever pneumonia in Birmingham, UK, in 1989, 88 (80%) of 110 patients for whom smoking data were available were current or ex-smokers, and only 22 (20%) had never smoked [19]. A subsequent case-control study in the same cohort demonstrated smoking to be a risk factor for Q fever [20]. Smoking was also shown to be an independent risk factor for Q fever infection in goat workers in rural Newfoundland [21], in 39 subjects with acute Q fever in southwest England and Northern Ireland [22], and in 103 patients with recent Q fever in a rural area of the Netherlands [2]. However, an extensive study conducted in Northern Ireland did not reach the same conclusions [23], as in other case-control studies conducted on subjects with ischaemic heart disease [12]. It should be noted that smoking appears as a Q fever risk factor only in studies based on epidemic outbreaks [2, 20–23]. This may be due to different epidemiological features involved in each case. Outbreaks occur in very specific places and at

specific times, thus the populations involved are more homogeneous than those included in seroprevalence studies. We did not find a significant association between the two variables in our sample.

We have observed that the majority of the control variables act as effect-modifying variables, such that the relationship is greater in their absence. These results are relevant, and could be linked to the known fact that some traditional risk factors, such as dyslipidaemia and diabetes, have a decreasing impact with advancing age [24]. In these circumstances, other lesser-known risk factors could act. Infections, for example, are more common in the elderly. This is due both to deterioration of the immune system associated with ageing, also called immunosenescence, and the associated co-morbidities [25]. In this sense, we currently know that *C. burnetti* can remain in the body for long periods of time [26], and be reactivated by immunosuppressive drugs or pregnancy [27]. As regards this fact, our group has recently demonstrated that a significant proportion of human abortions occurring in our area could be due to reactivation of Q fever [28]. Something similar could happen in elderly people. The persistence or reactivation of Q fever, even at a sub-clinical level, could be establishing a low-grade persistent inflammatory process, accelerating the arteriosclerosis process and precipitating cardiovascular events.

In summary, this study is the first to report a link between serological markers of past Q fever, measured late in life, and cardiovascular disease. However, it should be investigated whether this is a cause-effect relationship, or is a relationship dependent on other variables. This finding is of potential importance, because Q fever may be susceptible to prevention [29].

Transparency Declaration

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References

1. Raoult D, Tissot-Dupont H, Foucault C *et al.* Q fever 1985–1998. Clinical and epidemiologic features of 1383 infections. *Medicine (Baltimore)* 2000; 79: 109–123.
2. Karagiannis I, Schimmer B, Van Lier A *et al.* Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiol Infect* 2009; 137: 1283–1294.
3. Brouqui P, Dupont HT, Drancourt M *et al.* Chronic Q fever. Ninetytwo cases from France, including 27 cases without endocarditis. *Arch Intern Med* 1993; 153: 6428–6432.
4. Wegdam-Blans MC, Vainas T, van Sambeek MR *et al.* Vascular complications of Q-fever infections. *Eur J Vasc Endovasc Surg* 2011; 42: 384–392.
5. Fournier PE, Casalta JP, Piquet P, Tournigand P, Branchereau A, Raoult D. *Coxiella burnetii* infection of aneurysms or vascular grafts: report of seven cases and review. *Clin Infect Dis* 1998; 26: 116–121.
6. Choussat R, Montalescot G, Collet J *et al.* Effect of prior exposure to *Chlamydia pneumonia, Helicobacter pylori,* or Cytomegalovirus on the degree of inflammation and 1-year prognosis of patients with unstable angina pectoris or non-Q-wave acute myocardial infarction. *Am J Cardiol* 2000; 86: 379–384.
7. Pierangeli L, Nuzzolo L, Calabro R, Villanti P. [Myocardial infarct in the presence of serum antibodies against Coxiella burnetii]. *Boll Soc Ital Cardiol* 1967; 12: 205–209.
8. Fernández-Ruiz M, López-Medrano F, Alonso-Navas F, Aguado JM. *Coxiella burnetii* infection of left atrial thrombus mimicking an atrial myxoma. *Int J Infect Dis* 2010; 14: 319–321.
9. Ender PT, Phares J, Gerson G *et al.* Association of Bartonella species and *Coxiella burnetii* infection with coronary artery disease. *J Infect Dis* 2001; 183: 831–834.
10. Lovey PY, Morabia A, Bleed D, Peter O, Dupuis G, Petite J. Long term vascular complications of *Coxiella burnetii* infection in Switzerland: cohort study. *BMJ* 1999; 319: 284–286.
11. Wildman M, Ayres JG. Long term vascular complications of *Coxiella burnetii* infection. Cardiovascular risk factors cannot be ignored. *BMJ* 2000; 320: 58–59.
12. McCaughey C, Murray LJ, McKenna JP *et al.* Lack of association between serological evidence of past *Coxiella burnetii* infection and incident ischaemic heart disease: nested case-control study. *BMC Infect Dis* 2005; 5: 61–63.
13. NgehJ N, Goodbourn C. *Coxiella burnetii* and atypical respiratory infectious burden in stroke. *Stroke* 2005; 36: 1356–1360.
14. Pascual-Velasco F, Montes M, Marimón JM, Cilla G. High seroprevalence of *Coxiella burnetii* infection in Eastern Cantabria (Spain). *Int J Epidemiol* 1998; 27: 142–145.
15. Laslett LJ, Alagona PJ, Clark BA *et al.* The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. *J Am Coll Cardiol* 2012; 60: 41–49.
16. Hart RG, Pearce LA, Miller VT *et al.* Cardioembolic vs. noncardioembolic strokes in atrial fibrillation: frequency and effect of antithrombotic agents in the stroke prevention in atrial fibrillation studies. *Cerebrovasc Dis* 2000; 10: 39–43.
17. Bisoendial RJ, Kastelein JJ, Levels JH *et al.* Activation of inflammation and coagulation after infusion of C-reactive protein in humans. *Circ Res* 2005; 96: 714–716.
18. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373–383.
19. Hawker JI, Ayres JG, Blair I *et al.* A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? *Commun Dis Public Health* 1998; 1: 1807–1812.
20. Ayres JG, Flint N, Smith EG *et al.* Postinfection fatigue syndrome following Q fever. *QJM* 1998; 91: 105–109.
21. Hatchette TF, Hudson RC, Schlech WF *et al.* Goat-associated Q fever: a new disease in Newfoundland. *Emerg Infect Dis* 2001; 7: 413–419.
22. Orr HJ, Christensen H, Smyth B *et al.* Case-control study for risk factors for Q fever in southwest England and Northern Ireland. *Euro Surveill* 2006; 11: 260–262.
23. McCaughey C, McKenna J, McKenna C *et al.* Human seroprevalence to *Coxiella burnetii* (Q fever) in Northern Ireland. *Zoonoses Public Health* 2008; 55: 189–194.
24. Wilson PW, Kannel WB. Hypercholesterolemia and coronary risk in the elderly: the framingham study. *Am J Geriatr Cardiol* 1993; 2: 56–61.

25. Agarwal S, Busse PJ. Innate and adaptive immunosenescence. *Ann Allergy Asthma Immunol* 2010; 104: 183–190.
26. Harris RJ, Storm PA, Lloyd A, Arens M, Marmion BP. Long-term persistence of *Coxiella burnetii* in the host after primary Q fever. *Epidemiol Infect* 2000; 124: 543–549.
27. Sidwell RW, Gebhardt LP. Studies of latent Q fever infections. 3. Effects of parturition upon latently infected guinea pigs and white mice. *Am J Epidemiol* 1966; 84: 132–137.
28. Quijada SG, Terán BM, Murias PS, Anitua AA, Cermeño JL, Frías AB. Q fever and spontaneous abortion. *Clin Microbiol Infect* 2012; 18: 533–538.
29. Gidding HF, Wallace C, Lawrence GL, McIntyrec PB. Australia's national Q fever vaccination program. *Vaccine* 2009; 27: 2037–2041.